Article

Resynchronization of estrus in beef cattle: Ovarian function, estrus and fertility following progestin treatment and treatments to synchronize ovarian follicular development and estrus

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Abstract — The objective was to optimize rebreeding of nonpregnant, previously inseminated beef cattle. In Experiment 1, 43 cows received a used intravaginal progesterone-releasing insert (IVPRI; Days 0–7) 12.3 d after ovulation and received concurrently no treatment, 100 μg gonadotropin releasing hormone (GnRH), 1 mg estradiol cypionate (ECP), or 150 mg progesterone. Emergence of a new ovarian follicular wave was most synchronous ($P < 0.0001$) in the GnRH group. In Experiment 2, 675 heifers were given GnRH or no treatment on Day 0, fed melengestrol acetate (MGA; 0.5 mg/head/d) from Days 0–5 (Day 0 = 13–14 d after timed insemination; TAI), given 0.5 mg ECP or nothing on Day 7, and reinseminated 6–12 h after onset of estrus. Estrus was more synchronous ($P < 0.05$) in heifers given GnRH versus no treatment on Day 0. In Experiment 3, 317 TAI heifers were resynchronized with either MGA or a used IVPRI with or without ECP on Day 7; estrus was more synchronous ($P < 0.05$) and pregnancy rates were higher (54.1% versus 39.2%, $P < 0.05$) in heifers given a used IVPRI than those fed MGA. For resynchronization of heifers, pregnancy rates were not significantly improved with GnRH treatment, but were higher with a used IVPRI than with MGA.

Résumé — Resynchronisation des œstrus chez les bovins de boucherie : fonction ovarienne, œstrus et fertilité à la suite d’un traitement aux progestatifs et de traitements visant à synchroniser le développement folliculaire ovarien et l’œstrus. L’objectif de cette étude était d’optimiser la reproduction de vaches de boucherie non gestantes après avoir été inséminées. Dans l’expérience 1, 43 vaches ont reçu un dispositif intravaginal usagé libérant de la progestérone (DILP; jours 0–7) 12,3 jours après l’ovulation et concurremment, aucun traitement, 100 μg de gonadolibérine (GnRH), 1 mg de cypionate d’oestradiol (ECP) ou 150 mg de progestérone. L’apparition d’une nouvelle vague folliculaire était plus synchronisée ($P < 0,0001$) dans le groupe GnRH. Dans l’expérience 2, 675 génisses ont reçu de la GnRH ou pas de traitement au jour 0, de l’acétate de mélengestrol dans l’alimentation (AMG; 0,5 mg/tête/j) des jours 0–5 (jour 0 = 13–14 jours après insémination systématique; 1S), 0,5 mg de ECP ou rien au jour 7 et ont été réinsiminées 6–12 h après le début de l’œstrus. L’œstrus était plus synchronisé ($P < 0,05$) chez les génisses ayant reçu de la GnRH comparé au groupe n’ayant pas reçu de traitement au jour 0. Dans l’expérience 3, 317 génisses IS ont été resynchronisées avec soit de l’AMG, soit un DILP usagé avec ou sans ECP au jour 7; l’œstrus était plus synchronisé ($P < 0,05$) et les taux de gestation plus élevés (54,1 % contre 39,2 %, $P < 0,05$) chez les génisses ayant reçu un DILP usagé que chez celles ayant reçu de l’AMG par voie alimentaire. Pour la resynchronisation des génisses, les taux de gestation n’étaient pas significativement améliorés avec le traitement à la GnRH mais étaient plus élevés avec le DILP usagé qu’avec l’AMG.

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Introduction

Highly synchronous returns to estrus and overt estrous behavior would facilitate reinsemination of cattle nonpregnant to timed-artificial insemination (TAI). Based on the results of previous resynchronization studies, synchrony of behavioral estrus and the proportion of nonpregnant cattle detected in estrus must be improved (1–4). However, treatments to synchronize ovarian follicular development and increase estrus detection rates must not jeopardize pregnancy to TAI.

Macmillan et al (5) reported that an intravaginal progesterone-releasing insert (IVPRI) releases progesterone for at least 15 d and that it could be used successfully twice in TAI protocols (6). Furthermore, melengestrol acetate (MGA) may be an acceptable alternative for the resynchronization of previously inseminated heifers. However, in 2 recent studies, the pregnancy rate was higher in cattle resynchronized with a used IVPRI than in those fed MGA for 7 d (3,4). As exogenous progestins are given to delay returns to estrus in animals in which luteal regression has occurred, MGA treatment could result in the development of a persistent follicle and reduced fertility following reinsemination. We speculated that reducing the duration of MGA treatment might reduce the incidence of persistent follicles and increase fertility to reinseminations.

Estradiol treatment has been shown to induce a new ovarian follicular wave in progestin-treated cattle, regardless of the stage of the estrous cycle at the time of treatment (7). Furthermore, a reduced dose of estradiol cypionate (ECP) at progestin withdrawal enhanced manifestation of estrous behavior and synchronous luteinizing hormone (LH) release and ovulation (8). However, estradiol treatment of previously inseminated heifers has been reported to reduce pregnancy rates to TAI (4,9). On the other hand, an injection of progesterone at IVPRI insertion (13 or 14 d after TAI) did not affect pregnancy rate to TAI, but reduced both the occurrence of behavioral estrus and conception rates in those nonpregnant heifers that were detected in estrus and reinseminated (4).

Gonadotrophin releasing hormone (GnRH) may be an alternative for synchronizing follicular wave emergence in cattle with unknown pregnancy status. In addition, it has been suggested that GnRH treatment during diestrus would result in most cattle having 3 follicular waves (10), which may increase fertility at the subsequent estrus (11). However, the administration of GnRH between Days 12 and 16 of the estrous cycle in cows has been reported to extend the length of that cycle (12).

The objectives of the present studies were as follows: 1) to evaluate the effects of a used IVPRI and treatment with ECP, GnRH, or progesterone during diestrus on ovarian follicular dynamics, corpus luteum (CL) function, estrus, and ovulation in beef cows; and 2) to determine whether these treatments, along with either MGA or a used IVPRI, would synchronize returns to estrus and increase estrus and conception rates in nonpregnant beef heifers resynchronized following TAI.

Materials and methods

Experiment 1

The estrous cycles of nonlactating, nonpregnant, crossbred beef cows (n = 43; 3 to 9-y old) were synchronized and the time of ovulation was determined by twice-daily ultrasonography. On Day 0 (12.3, s = 0.7 d after ovulation), all cows received a once-used IVPRI (CIDR-B; Bioniche Animal Health Canada, Belleville, Ontario) that had been washed and disinfected as previously described (6). The cows were randomly allocated to receive 1 of the following concurrently: no further treatment (Control; n = 10); 1 mg of estradiol cypionate (ECP; Pfizer Canada, Kirkland, Quebec; n = 11); 100 μg of gonadotrophin releasing hormone (GnRH; Cystorelin, Merial Canada, Victoriaville, Quebec; n = 11), or 150 mg of progesterone (Sigma Chemical, St. Louis, Missouri, USA) in 2 mL of canola oil (n = 11). The ECP, GnRH, and progesterone treatments were all given IM. On Day 7, IVPRI were removed from all cows, and approximately half of the cows in each group received 0.5 mg of ECP, IM. Cows were monitored once daily from Days 0 to 8 by ultrasonography (Aloka SSD 500 with a 7.5 MHz linear-array transducer; ISM, Edmonton, Alberta) to determine ovarian follicular dynamics, and twice daily from Days 8 to 12 to determine the time of ovulation. The diameter of the CL and all follicles ≥ 3 mm were measured and recorded (13). The day of emergence of a follicular wave was defined as the day on which the dominant follicle was first identified at a diameter of 4 mm (14). When the follicular wave emergence did not occur during the observation period, the day of follicular wave emergence prior to treatment was estimated from the size of the dominant follicle at the time of treatment and a dominant follicle growth rate of 1.2 mm/d (15). Ovulation was confirmed by the disappearance of a large (> 10 mm) follicle that had been detected at the previous examination. Visual observation for estrous behavior was done for 40 min, 3× daily, from Days 8 to 12, but the cows were not inseminated. Every 24 h (from Days 1 to 9), blood samples were collected (by coccygeal venipuncture) into an evacuated tube containing heparin. Samples were centrifuged at 1500 × g for 20 min and the plasma was separated and stored at −20°C until assayed for progesterone by an enzyme-immunoassay (16). The intra- and inter-assay coefficients of variation were 7.1% and 10.2%, respectively.

Experiment 2

Angus and Angus-cross heifers (n = 675), 12- to 15-mo old and weighing approximately 320 to 400 kg, were used between April and June. All heifers were managed in 8 pens in a drylot, fed a barley-silage-based diet and synchronized for TAI with an MGA-based protocol. Thirteen or 14 d after TAI, heifers were fed melengestrol acetate (MGA; Pfizer Canada), 0.5 mg/head/d for 6 d (designated Days 0 to 5). On Day 0, heifers were randomly allocated to 1 of 2 groups to receive either no further treatment (control) or 100 μg of GnRH, IM. On Day 7 (2 d after the last feeding of MGA), approximately half of the heifers in each group received 0.5 mg of ECP, IM, in a 2 × 2 factorial design. Estrus was detected as in Experiment 1. Heifers observed in estrus were reinseminated 6 to 12 h later by the same technician, using commercial frozen-thawed semen from 1 of 2 bulls, balanced across treatment groups. Ultrasonographic pregnancy diagnosis was conducted approximately 28 d after TAI in heifers not seen in estrus following resynchronization, and approximately 55 d after the 2nd artificial

Table 1. Mean day of ovarian follicular wave emergence, and diameter of the dominant follicle at IVPRI removal and prior to ovulation in beef cows given an IVPRI and concurrently treated with nothing (control), 1 mg of estradiol cypionate (ECP), 100 μg of gonadotropin releasing hormone (GnRH) or 150 mg of progesterone (P4) on Day 0 (12.3, $s = 0.7$ d after ovulation). At IVPRI removal (Day 7), approximately half of the cows in each group received 0.5 mg of ECP (Experiment 1)

	Control	ECP	GnRH	P4
Number of cows	10	11	11	11
Day of follicular wave emergence	0.2	0.7	0.8	-0.2
s^1	3.7^x	4.1^x	0.8^y	3.3^x
Range (d)	-5 to 6	-6 to 5	-1 to 2	-6 to 5
Diameter (mm) of dominant follicle at:				
IVPRI removal (Day 7)	14.4	12.4	13.1	14.3
s^2	4.3^x	3.2^x	1.4^y	2.7^x
Range (d)	7 to 20	8 to 19	12 to 17	10 to 19
Just prior to ovulation	17.7^a	15.3^b	15.6^b	16.4^{ab}
s	2.6	2.0	1.4	2.5
Range (d)	14 to 21	13 to 20	14 to 18	13 to 21

[ab] means within the row tended to differ ($P < 0.08$)
s = standard deviation
$s^{1\ xy}$ standard deviations within a row, without a common superscript differed ($P < 0.0001$)
$s^{2\ xy}$ standard deviations within a row, without a common superscript differed ($P < 0.01$)
IVPRI = intravaginal progesterone-releasing insert

insemination (AI) in those that were detected in estrus and reinseminated.

Estrus rate was defined as the proportion of heifers detected in estrus out of those showing estrus, plus those subsequently diagnosed nonpregnant to TAI. Conception rate was defined as the proportion of heifers pregnant to reinsemination out of those observed in estrus and reinseminated. Pregnancy rate was defined as the proportion of heifers pregnant out of those synchronized for TAI, or the proportion of heifers pregnant out of those detected in estrus and reinseminated, plus those diagnosed nonpregnant to TAI.

Experiment 3

Angus and Angus-cross heifers ($n = 317$) were managed in 2 pens, fed, synchronized, and submitted to TAI, as described for Experiment 2. On Day 0 (13 or 14 d after TAI), heifers were randomly allocated either to be fed MGA for 6 d (as in Experiment 2) or to receive a once-used IVPRI for 7 d (as in Experiment 1). On Day 7, the IVPRIs were removed and approximately half of the IVPRI- and MGA-treated heifers were given 0.5 mg of ECP, IM. Estrus detection, reinsemination, and pregnancy diagnosis were done as in Experiment 2.

Statistical analyses

Throughout this manuscript, data are reported as means and standard deviations. Probability values ≤ 0.05 were considered significant, whereas $P \leq 0.1$ was considered a tendency. All data were analyzed with a statistical software program (SAS Version 8.2 for Windows; SAS Institute, Cary, North Carolina, USA) (17).

A one-way analysis of variance (ANOVA) was used to compare intervals from treatment to follicular wave emergence, and from IVPRI removal to estrus and ovulation among experimental groups in Experiment 1, and from TAI to reinsemination (Experiments 2 and 3). Means were compared with a protected least significant difference (LSD) test and equality of variances was compared by Bartlett's test. In Experiment 1, the number of follicles by day was categorized according to follicle size: small (3 to 5 mm), intermediate (6 to 9 mm), and large ($\geq$ 10 mm). The number of follicles in each size category was analyzed by using generalized estimating equations (GEE; PROC GENMOD). The main-effects model was assessed for first-order interactions where treatment and day remained in the model, with $P < 0.05$ for both variables in the GEE analysis. Data for CL and follicle diameters and plasma progesterone concentrations were analyzed by ANOVA for repeated measures (Proc Mixed Model), using autoregressive-1 (AR-1) as the covariate structure for repeated measurements (18). In Experiments 2 and 3, associations between pregnancy status to TAI and reinsemination and treatment groups were analyzed using a GEE (PROC GENMOD) method to account for clustering due to pen effects. Model specifications included a binomial distribution, logit link function, repeated statement with subject equal to pen, and an exchangeable correlation structure. Treatments were analyzed as a fixed effect with 2 categories. Proportional data were compared with the chi-square test.

Results

Experiment 1

Ovarian follicular wave emergence following treatment on Day 0 (12.3, $s = 0.7$ d after ovulation) was most synchronous ($P < 0.0001$), and diameter of the dominant follicle at IVPRI removal on Day 7 was least variable ($P < 0.01$) in the GnRH group (Table 1). The largest follicle present on Day 0 subsequently ovulated (after IVPRI removal) in 50.0%, 36.4%, 18.2%, and 54.5% of cows in the control, ECP-, GnRH-, and progesterone-treated groups, respectively ($P > 0.3$). Ovulation occurred 48 h after GnRH treatment in 8 of 11 cows; a new

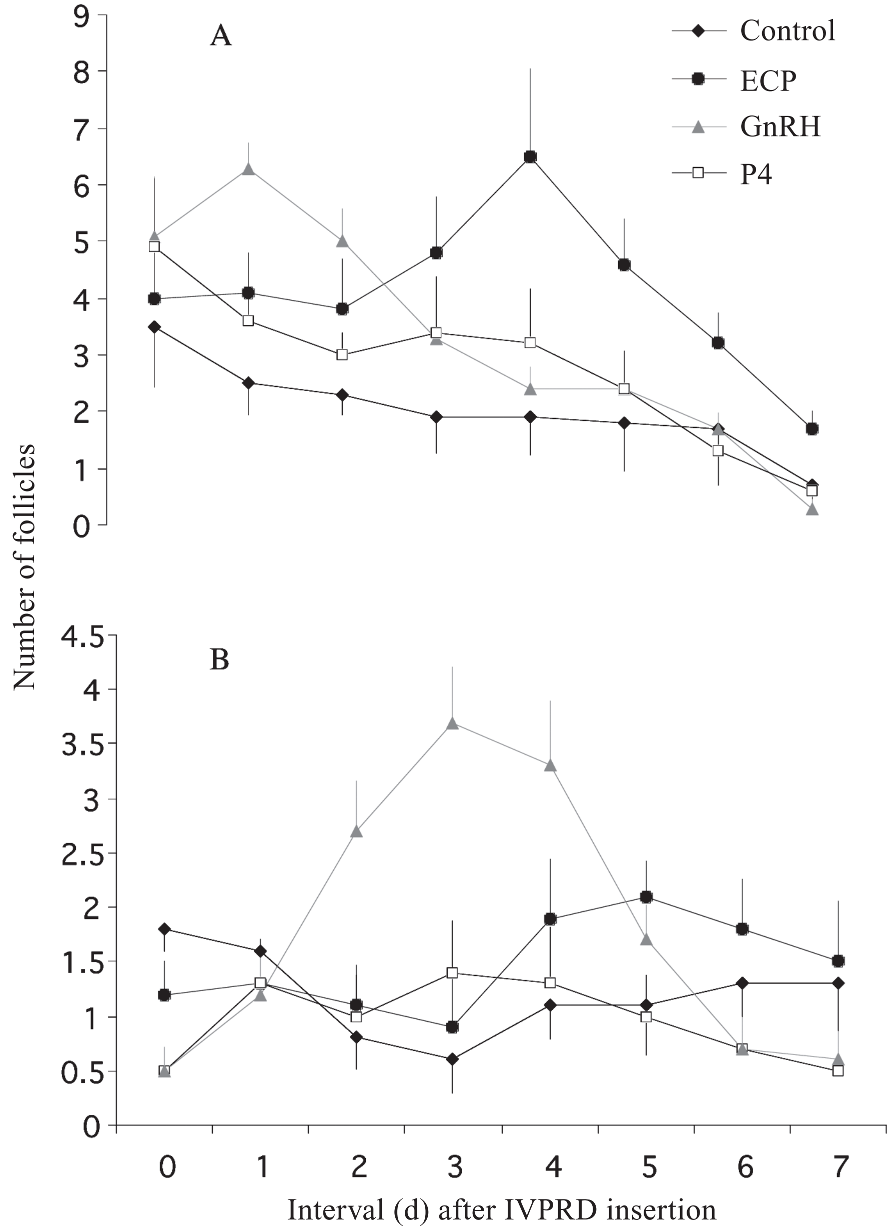

Figure 1. Numbers of small follicles (3 to 5 mm; A) and intermediate follicles (6 to 9 mm; B) in beef cows given an IVPRI and concurrently treated with nothing (control), 1 mg of estradiol cypionate (ECP), 100 μg of gonadotropin releasing hormone (GnRH) or 150 mg of progesterone (P4) 12.3, $s = 0.7$ d after ovulation (designated as Day 0). The number of small follicles was affected by day ($P < 0.0001$) and treatment ($P < 0.07$). The number of intermediate follicles tended to be affected by day ($P < 0.08$) and a treatment by day interaction ($P < 0.06$).

follicular wave emerged 24 h before ovulation in 7 of these 8 cows and in 1 cow, the largest subordinate follicle became the dominant follicle of the new wave.

The number of small follicles (3 to 5 mm) was affected by day ($P < 0.0001$) and tended to be affected by treatment ($P < 0.07$; Figure 1). The number of intermediate-sized follicles (6 to 9 mm) tended to be affected by day ($P < 0.08$) and there tended to be a treatment by day interaction ($P < 0.06$). There were no significant main effects or interactions for large follicles (> 10 mm). The diameter of the CL was affected by day ($P < 0.0001$) and tended to be affected by treatment ($P < 0.1$); there also tended to be a treatment by day interaction ($P < 0.06$). However, only day affected plasma progesterone concentrations ($P < 0.0001$).

Intervals from IVPRI removal to estrus and ovulation were not affected by treatments on Day 0 ($P < 0.2$; Table 2). However, 10/43 cows were not detected in estrus after IVPRI removal (3 received ECP and 7 received no treatment on Day 7; $P < 0.1$). The interval from IVPRI removal to ovulation tended to be affected ($P < 0.07$) by the administration of ECP at IVPRI removal (68.7, $s = 9.2$ versus 75.0, $s = 12.2$ h for ECP and control groups, respectively) and was affected ($P < 0.05$) by the interaction between treatments at IVPRI insertion and removal. Cows given either GnRH or ECP at IVPRI insertion

Table 2. Estrus and ovulation rates and mean intervals from IVPRI removal to estrus and ovulation in beef cows given an IVPRI and concurrently treated with nothing (control), 1 mg of estradiol cypionate (ECP), 100 µg of gonadotropin releasing hormone (GnRH) or 150 mg of progesterone (P4) on Day 0 (12.3, $s = 0.7$ d after ovulation). At IVPRI removal (Day 7), approximately half of the cows in each group received 0.5 mg of ECP (Experiment 1)

	Control	ECP	GnRH	P4
Estrus				
No ECP on Day 7				
Number of cows	2/5	3/5	5/6	4/5
Mean Interval (h)	32.0	50.7	48.0	35.0
s	0.0	4.6	11.3	10.0
Range	32	48 to 56	38 to 64	24 to 48
ECP on Day 7				
Number of cows	4/5	5/6	5/5	5/6
Mean Interval (h)	40.0	35.2	40.0	36.8
s	9.2	7.7	11.3	9.1
Range	32 to 48	28 to 48	32 to 56	28 to 56
Ovulation[1]				
No ECP on Day 7				
Number of cows	4/5	5/5	6/6	5/5
Mean Interval (h)	66.0[a]	81.6[b]	82.0[b]	67.2[a]
s	6.9	10.0	14.0	6.6
Range	60 to 72	72 to 96	72 to 108	60 to 72
ECP on Day 7				
Number of cows	5/5	6/6	5/5	5/6
Mean Interval (h)	69.6[a]	68.0[a]	67.2[a]	70.0[a]
s	10.0	9.8	10.7	9.3
Range	60 to 84	60 to 84	60 to 84	60 to 84

[1] Interval from IVPRI removal to ovulation; effect of treatment at IVPRI insertion ($P < 0.2$), effect of treatment at IVPRI removal ($P < 0.07$) and their interaction ($P < 0.05$)
[ab] Cows given either ECP or GnRH at IVPRI insertion and no further treatment at IVPRI removal had a significantly longer intervals from IVPRI removal to ovulation ($P < 0.05$)
s = standard deviation
IVPRI = intravaginal progesterone-releasing insert

and no further treatment at IVPRI removal had a longer interval from IVPRI removal to ovulation than cows in the other 6 groups (Table 2). One cow in the control group and 1 cow in the progesterone-treated group did not ovulate by Day 12, and data for end points that involved ovulation were excluded from the statistical analysis.

Experiment 2

Pregnancy rate to TAI was 37.9% and was not affected by treatments on Day 0 ($P < 0.7$) or Day 7 ($P < 0.3$). Overall, 419 heifers were diagnosed not pregnant to TAI. Treatment with GnRH on Day 0 had no effect on estrous rate ($P < 0.5$), whereas treatment with ECP on Day 7 tended to increase ($P < 0.1$) estrous rate (69.6% versus 62.7%; Table 3). Conception and pregnancy rates to reinsemination were not significantly different among treatment groups (Table 3).

Experiment 3

Pregnancy rate to TAI was 38.5% and was not affected by treatments on Day 0 ($P < 0.5$) or Day 7 ($P < 0.2$). Overall, 195 heifers were diagnosed not pregnant to TAI (Table 4). There was no effect of treatment on estrous rate (overall, 73.8%; $P < 0.4$). However, there was an effect of treatment on the interval from IVPRI removal on Day 7 to reinsemination (Table 4). Furthermore, heifers receiving an IVPRI tended ($P < 0.1$) to have a higher conception rate (68.8% versus 56.7%) and had a higher pregnancy rate (54.1% versus 39.2%; $P < 0.04$) than those fed MGA. Treatment with ECP on Day 7 had no effect on conception ($P < 0.4$) or pregnancy ($P < 0.8$) rates.

Discussion

In cattle resynchronized with progestins, fertility could be compromised by prolonged growth and dominance of the ovulatory follicle in those that had spontaneous luteal regression prior to progestin withdrawal. In that regard, fertility has been reported to be lower in heifers resynchronized with only a used IVPRI than in untreated controls (3,4). However, this could be overcome by inducing a new follicular wave at the start of progestin treatment. In the present study, GnRH was the only treatment that effectively induced follicular wave emergence in IVPRI-treated, diestrus cows (12.3, $s = 0.7$ d after ovulation). Although treatment with a GnRH agonist on Day 11 of the estrous cycle in an earlier study (12), resulted in increased estrous cycle length (primarily by reducing the frequency of cycles < 20 d in length), treatment with either GnRH (19) or human chorionic gonadotropin (hCG) (20) during diestrus in more recent studies did not affect estrous cycle length. In the current study, GnRH treatment 13 or 14 d after TAI induced ovulation and emergence of a new follicular wave in $> 80\%$ of cows, but it did not alter estrous cycle length, suggesting that GnRH maybe useful for resynchronization of cattle following TAI.

In at least 1 study, administration of GnRH during diestrus significantly increased the proportion of cattle with 3-waves (10), and results from at least 1 study have suggested that cattle with 3 follicular waves have higher fertility than those with 2 follicular waves (11). Therefore, treatments that result in all cattle having 3 follicular waves could increase fertility. However, GnRH treatment in Experiment 2 did not affect the pregnancy rate following TAI and did not increase the conception rate in reinseminated heifers, even though the synchrony of estrus suggested that GnRH may have synchronized follicular wave emergence. This study should be repeated using cows, as they are more likely than heifers to ovulate following GnRH treatment during diestrus (21,22).

Administration of either 1 mg of ECP or 150 mg of progesterone during diestrus in Experiment 1 did not effectively synchronize follicular wave emergence (relative to the control group). Circulating FSH concentrations and LH pulse amplitude have both been shown to decrease following administration of a combination of estradiol and progesterone (7,23), resulting in the regression of both FSH- and LH-dependent follicles (7). However, administration of 1 mg of ECP in IVPRI-treated cows has been reported to result in relatively asynchronous follicular wave emergence (8). Since circulating progesterone concentrations affect LH pulse frequency (24), progesterone treatment was expected to affect only LH-dependent follicles (25) (dominant follicles ≥ 9 mm in diameter [26]). Although all cows had a dominant follicle ≥ 9 mm in diameter on Day 0 in Experiment 1, injection of 150 mg progesterone (and insertion of a used IVPRI) was associated with emergence of a new follicular wave in only 45.5% of cows. In other studies, administration of an IVPRI for 24 h or injection of 100 or 200 mg progesterone in

Table 3. Reproductive outcome of nonpregnant heifers resynchronized with MGA starting 13 or 14 d (Day 0) after timed-artificial insemination (AI) (TAI). All heifers ($n = 675$) were fed MGA (0.5 mg/head/d) for 6 d and were allocated to receive 100 μg of GnRH or nothing on Day 0 and 0.5 mg of ECP or nothing on Day 7 (2 × 2 factorial design; Experiment 2)

	Control	GnRH	Control/ECP	GnRH/ECP
Number of heifers nonpregnant to TAI	108	107	101	103
Resynchronization Number in estrus	67	68	74	68
Estrous rate (%)[1]	62.0[b]	63.5[b]	73.3[a]	66.0[b]
Interval — Day 7 to reinsemination (d)	2.1, $s = 1.4^x$	2.3, $s = 1.2^y$	2.1, $s = 1.4^x$	1.9, $s = 1.1^y$
Number of pregnant to reinsemination	30	35	39	27
Conception rate (%)	44.8	51.5	52.7	39.7
Pregnancy rate (%)[2]	27.8	32.7	38.6	26.2

[xy] Standard deviations within a row, without a common superscript differed ($P < 0.05$)
[1] Treatment with ECP on Day 7 tended to increase estrous rate ($P < 0.1$)
[2] Based on numbers of heifers subsequently found to be not pregnant to TAI
MGA = melengestrol acetate
GnRH = gonadotropin-releasing hormone
ECP = estradiol cypionate

Table 4. Reproductive outcome in nonpregnant heifers resynchronized with MGA or a used IVPRI starting 13 or 14 d (Day 0) after timed-artificial insemination (TAI). Heifers ($n = 317$) were given MGA (0.5 mg/head/day) for 6 d or a once-used IVPRI for 7 d and were subsequently given 0.5 mg of ECP or nothing on Day 7 (Experiment 3)

	MGA	MGA/ECP	IVPRI	IVPRI/ECP
Number of heifers nonpregnant to TAI	56	41	51	47
Resynchronization number in estrus	38	29	37	40
Estrous rate (%)	67.8	70.7	72.5	85.1
Interval — Day 7 to reinsemination (d)	2.6, $s = 1.3^y$	2.4, $s = 1.4^y$	2.2, $s = 0.7^x$	2.6, $s = 1.3^y$
Number pregnant to reinsemination	22	16	28	25
Conception rate (%)	57.9[a]	55.2[a]	75.7[b]	62.5[b]
Pregnancy rate (%)[1]	40.4[c]	39.0[c]	54.9[d]	53.2[d]

[xy] Standard deviations within a row, without a common superscript differed ($P < 0.003$)
[ab] Percentages within a row, without a common superscript tended to differ ($P < 0.1$)
[cd] Percentages within a row, without a common superscript differed ($P < 0.04$)
[1] Based on numbers of heifers subsequently found to be not pregnant to TAI
MGA = melengestrol acetate
IVPRI = intravaginal progesterone-releasing insert
ECP = estradiol cypionate

cows with persistent follicles resulted in relatively synchronous emergence of a new follicular wave (27,28), suggesting that persistent follicles may be more sensitive to short-term changes in LH pulse frequency than "healthy" dominant follicles.

Resynchronization of estrus in cattle must not disrupt pregnancy from a previous insemination. In this regard, the use of estradiol to induce follicular wave emergence or estrus is of some concern in cattle with unknown pregnancy status. However, there are conflicting reports regarding the effects of estradiol in previously inseminated cattle. There were no apparent effects on pregnancy rate in cows given 1 mg of estradiol benzoate (3,29,30) or in heifers and cows given 0.5 or 1 mg of estradiol cypionate, respectively (3,30), to synchronize follicular wave emergence. Similarly, pregnancy rate was apparently unaffected when cows were given 1 mg of estradiol benzoate (3), heifers were given 0.5 mg of estradiol cypionate (3), or heifers and cows were given either 0.5 or 1 mg of estradiol cypionate (3,30) to induce estrus. In contrast, administration of 1 mg of estradiol benzoate to synchronize follicle wave emergence (9) or 0.5 mg estradiol-17β to resynchronize estrus (4) in beef heifers seemed to be associated with reduced pregnancy rates to the TAI. Although pregnancy rates following TAI in Experiments 2 and 3 were somewhat lower than expected, there was no indication that estradiol treatments were responsible. In addition, CL diameter and plasma progesterone concentrations were not affected by estradiol treatment in Experiment 1. Whether reduced doses of estradiol will induce luteolysis or estrus in pregnant heifers needs further study.

Follicle numbers tended to be affected by treatment at IVPRI insertion in Experiment 1. Numbers of small follicles increased 1 and 4 d after treatment in GnRH- and ECP-treated cows, respectively, but declined in cows given progesterone. Although GnRH induced ovulation between 24 and 48 h after treatment in 8 of 11 cows, the increase in the number of small follicles before ovulation was probably due to increased concentrations of FSH following GnRH treatment (31) rather than ovulation of the dominant follicle (32). Indeed, emergence of a new follicular wave after ovulation occurred in only 1 GnRH-treated cow. However, ovulation or atresia of the dominant follicle is necessary to sustain the growth of FSH-dependent follicles (33). In GnRH-treated cows, the number of medium-sized follicles increased between Days 2 and 4 (peak on Day 3), which may reflect follicles emerging as a result of an earlier, transient GnRH-induced increase in FSH. In this regard, Macmillan and Thatcher (19) reported that a single injection of a GnRH agonist on Day 12 increased the number of medium-sized follicles 1 to 6 d later.

High rates of both synchronous estrus and conception are needed to optimize pregnancy rates in nonpregnant cattle returning to estrus following AI. In previous studies that used only progestins to resynchronize estrus, there was a clear need to increase the number of nonpregnant cattle displaying estrus after progestin removal (2–4). Insertion of a used IVPRI in 2 different studies (2,4) resulted in 60% to 80% of the non-pregnant heifers being detected in estrus over a 5-day interval, whereas the proportion of non-pregnant heifers that returned to estrus following resynchronization with MGA was 65.9% (3). Although estrus was detected by visual observation in most previous studies, the proportion of nonpregnant cattle detected in estrus was not greatly improved when an electronic, mount-detecting system was used (3,4). Therefore, we hypothesized that the inclusion of 0.5 mg of ECP at the time of progestin removal would increase the proportion of nonpregnant cattle observed in estrus. Indeed, treatment with ECP on Day 7 in MGA-treated heifers in Experiment 2 tended ($P < 0.1$) to increase estrous rate (69.6% versus 62.7%). However, there was no significant effect of ECP treatment on estrous rate in Experiment 3 and no significant effect of ECP on pregnancy rate (the product of estrus detection and conception rate) in Experiments 2 or 3. Therefore, we concluded that there was no benefit to giving ECP after progestin withdrawal in these experiments. As this treatment could also result in the expression of estrus in pregnant animals, it can not be recommended.

Overall, conception and pregnancy rates were higher in nonpregnant heifers resynchronized following TAI with a used IVPRI as compared with those fed MGA (Experiment 3). We have previously reported that conception and pregnancy rates were lower in heifers resynchronized with MGA for 7 d (49.6% and 40.4%, respectively) than in those given a used IVPRI for 7 d (65.1% and 61.4%, respectively) or in untreated controls (62.2% and 54.9%, respectively) (4). In the present study, a shorter-term MGA treatment regimen and an attempt to synchronize ovarian follicular dynamics did not improve fertility in MGA-fed heifers. In Experiment 2, administration of GnRH at the start of MGA treatment improved the synchrony of estrus, but it did not improve conception rate. Stevenson et al (3) also reported that administration of 0.5 mg ECP at first feeding and after cessation of MGA feeding (13 and 20 d after AI, respectively) did not increase conception rates over untreated controls. Although treatment with estradiol-17β and progesterone at the start of MGA feeding on Day 13 increased conception rates by 14.0% (relative to heifers receiving only MGA) in our previous study (4), the increased conception rates were still 11% lower than in untreated controls and 20.0% lower than in heifers treated with a used IVPRI plus estradiol-17β and progesterone. Based on present and previous studies, it is clear that MGA is not effective for the resynchronization of heifers following TAI.

In an earlier study (4), the use of a previously used IVPRI following TAI to resynchronize returns to estrus resulted in a reduced period of estrus detection, but we were concerned that conception rates may have been adversely affected. The conception rate was numerically lower in the resynchronized heifers than in untreated control heifers (62.5% versus 76.7%), but the difference was not significant, perhaps due to limited statistical power to detect a difference (4). Stevenson et al (3) also reported lower conception rates in heifers receiving a used IVPRI to resynchronize estrus (33.3%) than in untreated controls (60.0%). However, the same has not been reported following the use of a new IVPRI; Macmillan and Peterson (1) reported conception rates of 65.6% in cows resynchronized with a new IVPRI, and Chenault et al (34) reported a conception rate of 26.7% in lactating dairy cows resynchronized with a new IVPRI, which was not different from that in untreated controls (30.9%). It is noteworthy that when once-used IVPRIs, were compared with new IVPRIs in a TAI protocol, no difference in pregnancy rates was observed (6); in fact, pregnancy rates were numerically higher in cattle synchronized with a once-used IVPRI.

The economic feasibility of using an IVPRI (new or used) for resynchronization of returns to estrus in cattle not pregnant to TAI will have to be determined on a case-by-case basis; variables to consider would include the pregnancy rate to TAI, the cost and practicality of estrus detection and animal handling, the availability of bulls for natural service, the value of pregnancies resulting from AI versus natural service, and the importance of date of conception. For example, if pregnancy rates to TAI were high, the cost-benefit of resynchronization may be limited. Unfortunately, the decision to resynchronize must be made prior to determination of pregnancy status to the TAI.

In summary, GnRH treatment during diestrus synchronized ovarian follicular wave emergence without affecting the length of the estrous cycle in nonbred beef cows, and shortened the estrus detection period in heifers resynchronized with progestins following TAI. In contrast, 1 mg of ECP or 150 mg of progesterone given at IVPRI insertion during diestrus did not synchronize follicular wave emergence. Although ECP treatment following withdrawal of MGA tended to increase the estrus detection rate, it did not significantly improve pregnancy rates to reinsemination. Resynchronization by feeding MGA resulted

ARTICLE

in lower conception and pregnancy rates than did a used IVPRI; giving GnRH at the start of MGA treatment did not improve fertility. It was noteworthy that none of the resynchronization protocols significantly affected CL function, estrous cycle length, or ongoing pregnancies to TAI.

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